From isolation of adult adipose tissue derived stem cells ADAS to labelling with superparamagnetic iron oxide nanoparticles: first approaches to unleash the potential.

Arnaud M. Capuzzo[1], Ilaria Scambi[2], Raffaella Mariotti[2], Pasquina Marzola[3]

[1]Department of Diagnostics and Public Health, University of Verona, Verona, Italy

[2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy

[3]Department of Informatic, University of Verona, Verona, Italy

The use of adult adipose-derived stem cells (ADAS) as a treatment for neurological diseases is in promising development. Extracellular vesicles such as exosomes (EXO), which impact surrounding cells, are the main biological agents of ADAS. Exosome localization and tracking techniques need to be effective and non-invasive in the current development of exosome therapies. Exosomes must be labeled with contrast agents, such as ferrous superparamagnetic nanoparticles (NPs). The current research project aims to validate the therapeutic efficacy of ADAS-derived EXOs labelled with different NPs in models of neurodegenerative diseases, capable of providing an imaging and cell therapy approach.

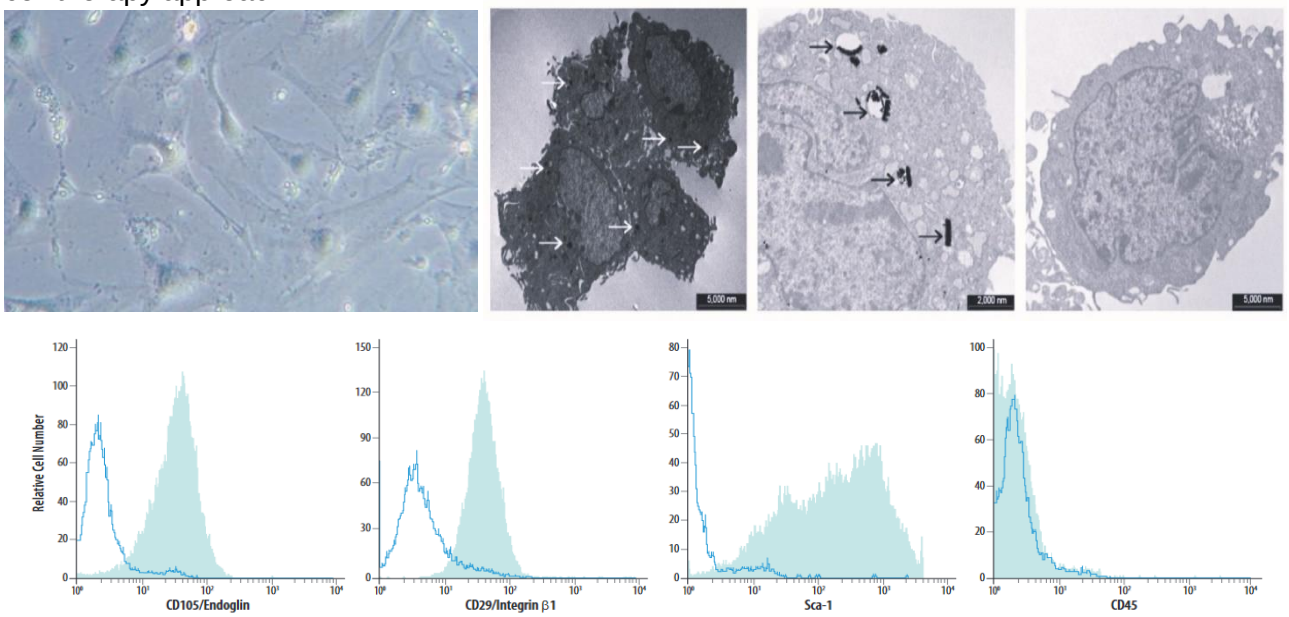

Figure Left. Adult adipose tissue derived murine stem cells ADAS. ADAS cultured with complete DMEM containing 10% FBS and 1% P/S mix 1:1 and incubated at 37°C in a 5% CO2 atmosphere. These cells have the characteristic of producing exosomes, which in recent studies have shown to have unique characteristics for some neuropathology's (Bonafede et al. 2020). *The image acquisition was done using a bright field optical microscope, Olympus BX-51 (Olympus, Tokyo, Japan) equipped with a digital camera (DKY-F58 CCD JVC, Yokohama, Japan) and connected with a PC endowed with Image-Pro Plus 7.0 software.*

Figure Right. TEM images of ADAS incubation with nanoparticles (NPs) were morphologically analysed through a Transmission Electron Microscope (TEM) in order to confirm the intracellular uptake of NPS and visualise their intracellular localization. The scale bar in the left and right pictures is 5,000 nm, and the centre picture is focused on the endocytic invagination containing nanoparticles and the internalised nanoparticles inside the endosome (scale bar 2,000 nm). Cell pellets were fixed for 1 h in 2% glutaraldehyde in 0.1 M phosphate buffer (PB) and, after washed, postfixed for 1 h in 1% OsO4 diluted in 0.2 M K3Fe(CN)6. After rinsing in 0.1 M PB, the samples were dehydrated in graded concentrations of acetone and embedded in a mixture of Epon and Araldite (Electron Microscopic Sciences, Fort Washington, PA, USA). Ultrathin sections were cut at 70 nm thickness on a Ultracut E ultramicrotome (Reichert-Jung, Heidelberg, Germany), placed on Cu/Rh grids and contrasted with lead citrate. Samples were observed with Pa Philips Morgagni 268 D electron microscope (Fei Company, Eindhoven, The Netherlands) equipped with a Mega View II camera to acquire digital images (Busato et al. 2017).

Figure Above. Verification of ADAS Cell Identity by Analysis of MSC Marker Expression. ADAS were stained with the indicated antibodies (filled histograms) or the corresponding isotype control (open histograms). ADAS demonstrate the characteristic expression of CD105/Endoglin, CD29/Integrin β1, Sca-1 while CD45 is a negative control.

Bonafede, Roberta, Ermanna Turano, Ilaria Scambi, Alice Busato, Pietro Bontempi, Federica Virla, Lorenzo Schiaffino, Pasquina Marzola, Bruno Bonetti, e Raffaella Mariotti. 2020. «ASC-Exosomes Ameliorate the Disease Progression in SOD1(G93A) Murine Model Underlining Their Potential Therapeutic Use in Human ALS». International Journal of Molecular Sciences 21(10):3651. doi: 10.3390/ijms21103651.

Busato, Alice, Roberta Bonafede, Pietro Bontempi, Ilaria Scambi, Lorenzo Schiaffino, Donatella Benati, Manuela Malatesta, Andrea Sbarbati, Pasquina Marzola, e Raffaella Mariotti. 2017. «Labeling and Magnetic Resonance Imaging of Exosomes Isolated from Adipose Stem Cells». Current Protocols in Cell Biology 75(1):3.44.1-3.44.15. doi: 10.1002/cpcb.23.